LYSIS OF FUNGI BY SOIL MICROORGANISMS AND FUNGICIDES INCLUDING ANTIBIOTICS[1]

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SUMMARY

Several species of fungi growing on agar media were lysed by the action of certain isolates of *Streptomyces* spp. from soil. Antibiotics and fungicides that inhibited growth of *Glomerella cingulata* also lysed mycelium of this fungus on peptone agar. Lytic zones produced by the colonies of *Streptomyces* spp. were usually clearer than those resulting from the action of antibiotics and fungicides. Some lytic zones produced by *Streptomyces* spp. were entirely free from mycelium of the test fungus. The *Streptomyces* spp. lysed both living and dead mycelium of *G. cingulata* in peptone agar, but antibiotics and fungicides lysed only living mycelium. Mycelium of *G. cingulata* growing in peptone broth was partially disintegrated when Acti-dione or isolate 55 of *Streptomyces* was added to the cultures.

Although there have been frequent reports of inhibition of fungi by microorganisms and by fungicidal chemicals, lysis of fungi has been observed infrequently. Disintegration of fungal mycelium by actinomycetes has been observed in culture (12, 15, 17), and several instances of lysis of fungi by bacteria are known (1, 6, 7, 9, 10, 11, 13). Lysis of fungal mycelium in contact with soil also has been observed, but the agents responsible for such action were not stated (2, 4, 5). Certain plant diseases have been controlled experimentally with mycolytic microorganisms (2, 5, 7, 10, 11, 13).

In work by Leben and Lockwood (unpublished data), contaminating microorganisms that sometimes appeared on agar plates used for assaying antifungal antibiotics were frequently surrounded by zones in which the mycelium of the test fungus had disintegrated. As a result of these observations, investigations were made of the lytic action on the fungus *Glomerella cingulata* (Ston.) Spauld. & Schrenk of various microorganisms and of several fungicides including antibiotics.

The modes of action of isolates of *Streptomyces* spp., of antibiotics, and of fungicides as agents of lytic activity are not known. In this paper, therefore, such terms as "lytic microorganism" and "lytic agent" are used when referring to microorganisms or substances that initiate lysis in fungi or are themselves the causal agents of such disintegration.

MATERIALS AND METHODS.—*Method of testing soil microorganisms and fungicides for lytic activity.*—Soil microorganisms used were obtained during the development of a method for isolating lytic microbes from soils (3). Microorganisms lytic to mycelium of *Glomerella cingulata*, *Colletotrichum circinans* (Berk.) Vogl., or *Helminthosporium sativum* Pam., King, & Bakke were employed.

All lysing organisms were members of the genus *Streptomyces*. They were cultured on yeast-extract agar (per liter: yeast extract, 2 g; glucose, 10 g; agar, 20 g) at 26°C. Sixteen antibiotics, primarily of an antifungal nature, and 5 other fungicides were

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used in the investigations. Each was dissolved in an appropriate solvent in concentrations of 1000, 500, 250, and 125 ppm of active material. The fungicides were filtered to remove any insoluble material present.

Agar tests for lytic activity were made in Petri plates on peptone agar (per liter: peptone, 5 g; agar, 20 g) containing a culture of the fungus *G. cingulata*. The culture was prepared by mixing a conidial suspension (containing approximately 20,000 spores per ml of water) with the warm agar at the rate of 1 to 5, respectively, and then incubating the mixture for 2–3 days. Chemicals or spore suspensions of the lytic isolates of *Streptomyces* spp. were applied to the surface of the agar in sterile blotting paper disks (diameter, 6 mm) that contained 0.02 ml of solution or suspension. Duplicate disks were prepared and placed on different plates. Chemicals were tested for inhibition of *G. cingulata* by a similar method except that disks containing the chemicals were applied immediately after plates were poured and the agar hardened. Pyridine, the solvent for ascosin, produced a narrow lytic zone; therefore disks containing pyridine were dried before being placed on the agar. Other solvents were neither lytic nor inhibitory.

For tests made in liquid media, 60 ml of peptone broth (per liter: peptone, 5g) were added to each of a series of 125-ml flasks. Ten ml of a conidial suspension of *G. cingulata* containing approximately 20,000 spores per ml was added to each of the flasks. After *G. cinculata* had grown for 5 days in stationary culture, either Acti-dione dissolved in ethanol or a spore suspension of isolate 55 of *Streptomyces* spp. was added to the cultures. Cultures were incubated for an additional 5 days. Dry weights of mycelium were determined at the time of addition of the lytic agents and, in treated and control flasks, at the end of the incubation period. Ethanol, at the highest concentration used (3500 ppm), did not lyse *G. cingulata*.

Photometric method for determining lysis.—A method similar to that used by Hollis (8) was devised for determining changes in apparent density of mycelium. The apparatus consisted of a microscope with substage condenser, diaphragm, and concave mirror but with eyepiece and objectives removed; a microscope lamp with condenser and diaphragm; and an electrophotometer (Photovolt model 200M). The apparatus was adjusted to give readings of 100 when a beam of light 4 mm in diameter at stage level was passed through petri plates with peptone agar only and readings of approximately 30 when the light was passed through agar that contained dense mycelium. It was necessary that all adjustments be kept constant. One reading was made for each zone each time data were taken, and all readings for a given zone were taken in the same position on the plate. An increase in the amount of light transmitted indicated that lysis had occurred. Lysis of mycelium of *G. cingulata* was perhaps greater than indicated by the photometric readings since the agar became more opaque as dehydration increased.

All experiments were repeated 1 or more times with similar results.

RESULTS.—*Evidence for lytic activity.*—Zones produced by the addition of chemicals or of certain isolates of *Streptomyces* spp. to agar containing a culture of the fungus *Glomerella cingulata* were studied by microscopic examination and with an electrophotometer. The mycelium in these zones appeared to be less dense when examined with the naked eye than did that in the agar surrounding the zones. Microscopic examination of mycelium in the zones was made by direct observation of the agar in the petri plates and of small pieces of agar cut from the zones and crushed on glass slides. Anilin blue or neutral red were sometimes used to stain mycelium. Hyphal threads from all lytic zones examined were less distinct and fewer in number than in control plates. No mycelium could be seen in zones produced by certain isolates of *Streptomyces* spp.

The amount of light transmitted through certain zones was compared with the relative amount of mycelium observed microscopically in the same zones. Those zones through which the most light was transmitted contained fewer and less distinct hyphae than did those through which the least light was transmitted.

Continued growth of the test fungus outside the zone following treatment of an agar culture with a chemical or with certain isolates of *Streptomyces* spp. made it difficult to determine whether the zone produced was merely the result of the prevention of additional growth of the fungus in the zones or was due to the actual dissolution of the mycelium. By use of the photometer, the amount of light transmitted through such zones was found to increase from day to day. This effect, therefore, was interpreted as lytic. The percentage light transmission through agar containing mycelium of *G. cingulata* that was not treated with chemicals or with isolates of *Streptomyces* spp. decreased daily until maximum growth was achieved.

An antifungal antibiotic, Acti-dione, and a *Streptomyces* isolate, No. 55, both of which appeared to lyse mycelium of *G. cingulata* in agar, were tested for their effect on 5-day-old mycelium of this fungus growing in peptone broth (Table 1). Five days after the addition of Acti-dione to broth cultures of the fungus, dry weights of mycelium from treated cultures were 30–51 per cent less than those of the original mycelium, whereas the dry weights of mycelium from untreated cultures were 23–24 per cent greater than those of the original mycelium. Isolate 55 of *Streptomyces* spp. caused a similar decrease in weight of mycelium. Apparently, the mycelium of *G. cingulata* was lysed by these agents.

Lytic activity of isolates of Streptomyces spp. and of fungicides.—Characteristics of the lytic zones produced by *Streptomyces* spp. were studied by streaking isolates on 2-day-old cultures of *G. cingulata* grow-

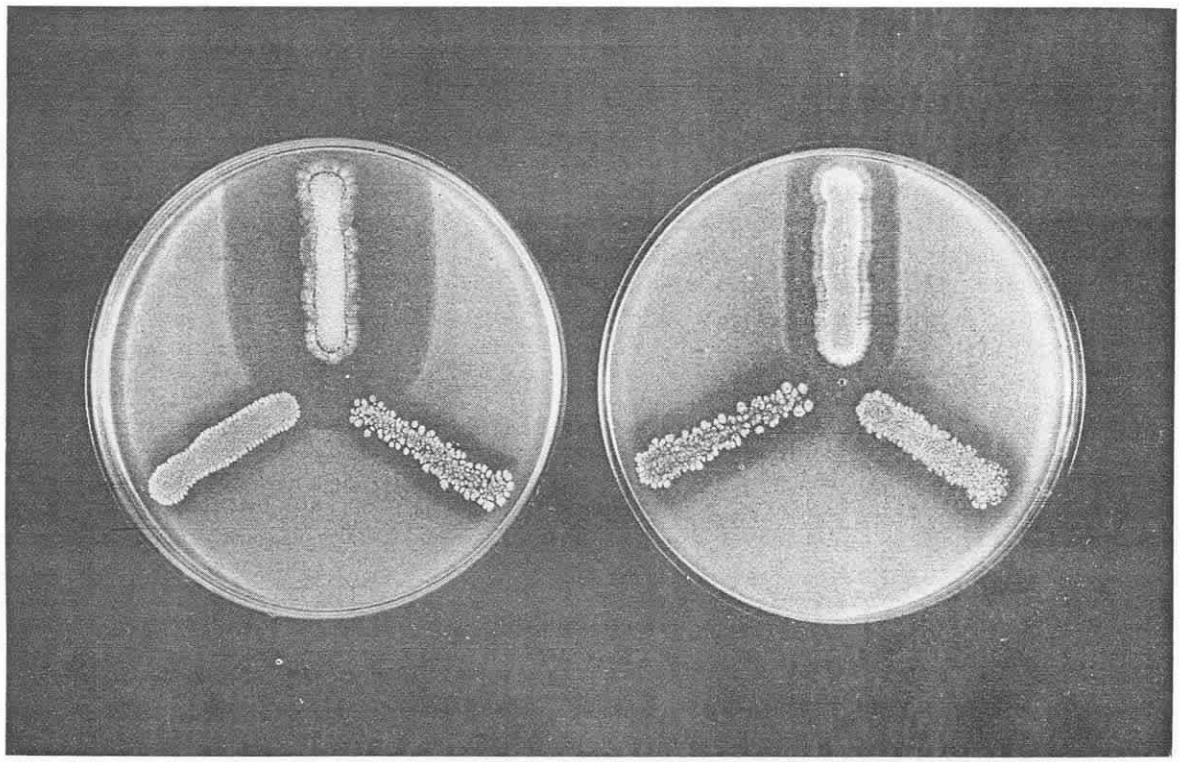

FIG. 1. Lytic zones produced by isolates of *Streptomyces* spp. streaked on peptone agar containing a culture of *Glomerella cingulata*.

ing on peptone agar (Fig. 1). Lytic zones developed well only on media low in nutrients that supported appressed growth of the test fungus (3). Detectable zones rarely developed on media that supported abundant aerial growth. Zones usually appeared 2–10 days after streaking. Different isolates varied greatly in size and clarity of zones produced as determined by visual examination. Occasionally, zones were observed that extended less than 1 mm from the colony of *Streptomyces* spp. Conversely, some colonies produced zones that covered the greater part of a petri plate. The same isolate always produced zones of the same relative size and clarity. In general, the narrower the zones the greater was their visual clarity, and any given zone appeared uniform throughout with respect to density of mycelium. In some zones no mycelium could be detected by microscopic examination of stained or unstained preparations. Zones produced by 2 different colonies of *Streptomyces* spp. in the same plate sometimes overlapped. These overlapped areas frequently contained less mycelium of the test fungus than did zones produced by single colonies. The above observations were confirmed by photometric and microscopic determinations. No direct parasitic action of the colonies of *Streptomyces* spp. on the mycelium of *G. cingulata* was observed.

TABLE 1.—*Lytic action of Acti-dione and of isolate 55 of Streptomyces spp. on mycelium of Glomerella cingulata in peptone broth*

Lytic agent	Concentration ppm	Percentage (by weight) of original mycelium remaining 5 days following addition of lytic agent to a 5-day-old culture[a]	
		Test 1	Test 2
Acti-dione[b]	125		70.3
Acti-dione	500	48.6	62.2
Acti-dione	1000	54.3	
Isolate 55		62.9[c]	43.2[c]
None		122.9	124.3

[a] Based on average of 4 cultures.
[b] 3-[2- (3,5-Dimethyl-2-oxocyclohexyl) -2-hydroxyethyl] glutarimide.
[c] Based on combined weight of mycelium of *G. cingulata* and isolate 55 of *Streptomyces* spp.

Diffusible substances apparently were produced by the lytic microorganisms. Disks of agar were taken from areas adjacent to colonies of lytic microorganisms growing on unseeded peptone agar. Following transfer of the disks to agar containing *G. cingulata*, zones that were identical to those that surrounded the lytic microorganisms themselves appeared around some of the disks.

Fungicides were applied to peptone agar containing 2-day-old cultures of *G. cingulata* and to peptone agar freshly seeded with spores of this fungus. From 1 to 5 days following application of chemicals to the fungus-agar substrates, zones surrounded most of the

disks. Of 21 chemicals tested, all except griseofulvin, mycophenolic acid, and neomycin inhibited growth in freshly seeded plates; the same chemicals also lysed *G. cingulata* when applied to 2-day-old cultures (Table 2). Frequentin and gladiolic acid produced narrow lytic zones on 2-day cultures but did not prevent growth of *G. cingulata* in freshly seeded plates. Lysis of *G. cingulata* by all chemicals except frequentin and gladiolic acid began within 24 hours after application; lytic activity by these 2 materials was not observed until 11 days after application. Lytic zones and zones of inhibition produced by the same chemical at a given concentration were of approximately equal diameters and clarity as determined visually. The replacement of assay disks with new disks containing the same fungicide did not increase the amount of light transmitted through the zones. Although lytic zones produced by antibiotics and by fungicides usually appeared sooner than did zones produced by *Streptomyces* spp., in many cases zones produced by the latter were more translucent.

Autolysis of *G. cingulata* in peptone agar was observed (see seeded control in Table 2). The amount of mycelium increased rapidly following application of conidial suspensions of *G. cingulata* to the agar, maximum density being obtained in 2–4 days. Light transmission through the agar then increased gradually for about 10 days to a point at which no further change occurred. The amount of mycelium that disintegrated as a result of autolysis was considerably less than the amount that disintegrated when *Streptomyces* spp. or chemicals were present.

Comparison of mode of action of Streptomyces spp. and of fungicides.—The fact that most of the antifungal chemicals tested lysed mycelium of *G. cingulata* suggested that lytic action of *Streptomyces* spp. was due to antibiotics which they produced. An attempt was made to determine if the modes of action of the chemicals and *Streptomyces* spp. were similar or different. Petri plates containing peptone agar in which *G. cingulata* was growing were exposed to chloroform vapor for 4 hours. Similar plates were autoclaved for 5 minutes at 10 lb./sq. in. Both methods killed *G. cingulata*. Assay disks containing fungicides and spore suspensions of lytic microorganisms were placed on the surface of the medium. Whereas *Streptomyces* spp. lysed both living and dead mycelium (Table 3), the chemicals lysed only living mycelium (Table 2). The action of *Streptomyces* spp. in lysing mycelium of *G. cingulata*, therefore, appeared to differ from that of the fungicides.

More light was transmitted through plates that contained mycelium killed by chloroform or by autoclaving than through plates in which the mycelium was not killed (Tables 2, 3). There appeared to be less mycelium in those plates that were exposed to chloroform than in those that were autoclaved. Chloroform vapor may exert lytic effects on fungi similar to those produced by other chemicals.

TABLE 2.—*Relative amount of lysis by antibiotics and by fungicides of living and dead mycelium of Glomerella cingulata in agar as determined by photometric method*

	Mean percentage light transmission[b]					
			Killed mycelium			
	Living mycelium		Autoclave		Chloroform	
Chemical[a]	0 Days[c]	15 Days[c]	0 Days[c]	15 Days[c]	0 Days[c]	15 Days[c]
Acti-dione	28	58	41	41	62	59
Antimycin A	30	57			60	59
Ascosin	29	70	38	38	64	62
Candicidin	26	60			62	62
Endomycin	29	68			63	62
Fradicin	25	55	39	37	62	60
Frequentin	27	50			63	65
Gladiolic acid	30	55			58	60
Gliotoxin	24	50			72	63
Griseofulvin	23	34			68	70
Helixin B	26	59			62	62
Mycophenolic acid	27	24			67	70
Mycostatin	30	78	44	45	61	61
Neomycin	25	30			65	63
Rimocidin	28	62			70	71
Thiolutin	27	75			59	63
Coromerc[d]	29	58			61	55
Ferbam[e]	30	70			71	68
Mercuric chloride	30	62			63	60
Thiram[f]	27	65	42	40	67	65
Zineb[g]	29	70			67	64
Seeded control	27	36	43	43	65	62
Unseeded control	100	91	100	92	100	90

[a] Appreciation is expressed to the following manufacturers and person for supplying chemicals used in these tests: Commercial Solvents Corp., Olin-Mathieson Corp., S. B. Penick and Co., Charles Pfizer and Co., and Dr. Curt Leben.

[b] A percentage light transmission of 23–30 indicates no lysis; one of 100 indicates complete lysis (or no fungus growth).

[c] Time after application of chemicals to 2-day-old cultures of *G. cingulata* in peptone agar.

[d] *N*-phenylmercuriethylenediamine.

[e] Ferric dimethyldithiocarbamate.

[f] Bis(dimethylthiocarbamoyl) disulfide.

[g] Zinc ethylenebis[dithiocarbamate].

DISCUSSION.—Although lysis of fungi in soils seldom has been reported (2, 4, 5), lytic activity of microorganisms in soils may be an important process in the ecology of soil microflora. It is well known that the population of soil microorganisms is influenced by such factors as soil depth, fertility, moisture, temperature, and cropping sequences (16), but the mechanisms bringing about changes in population are not well understood. Many isolates of *Streptomyces* spp. obtained from soil lysed fungal mycelium growing in a medium deficient in nutrients. This suggests that lytic action may occur in soils, most of which are similarly deficient in nutrients for optimum fungus growth. Furthermore, the capacity to lyse fungus mycelium may confer a competitive advantage on the lytic microbes, for it is possible that the metabolites released by lytic action are utilized by the lytic micro-

TABLE 3.—*Relative amount of lysis by isolates of Streptomyces spp. of living and dead mycelium of Glomerella cingulata in agar as determined by photometric method*

Streptomyces isolate	Mean percentage light transmission[a]					
	Living mycelium		Killed mycelium			
			Autoclave		Chloroform	
	0 Days[b]	15 Days[b]	0 Days[b]	15 Days[b]	0 Days[b]	15 Days[b]
Isolate 3	24	46			64	70
Isolate 12	24	77	51	75	65	80
Isolate 22	32	66				
Isolate 33	36	79			66	80
Isolate 41	29	57			60	75
Isolate 49	31	61	47	74		
Isolate 55	33	78	38	59		
Isolate 56	26	54			62	80
Isolate 64	26	65				
Isolate 69	32	71				
Isolate 76	31	78			60	74
Seeded control	27	36	43	43	65	62
Unseeded control	100	91	100	92	100	90

[a] A percentage light transmission of 24–36 indicates no lysis; one of 100 indicates complete lysis (or no fungus growth).

[b] Time after transferring isolates of *Streptomyces* spp. to 3-day-old cultures of *G. cingulata* in peptone agar.

organisms in growth processes.

Since antibiotics are produced by many species of *Streptomyces*, it might be inferred that lytic action of these isolates was due to antibiotics that they produced. This may be true in part, but *Streptomyces* spp. lysed both living and dead mycelium whereas antibiotics lysed only living mycelium, an indication that other substances also were produced. Saltan (14) observed disintegration of dead bacterial cells by *Streptomyces* spp., presumably as a result of enzymatic action. Enzymes synthesized by lytic microorganisms may, in addition to the antibiotics, be responsible for the disintegration of the mycelium of fungi. The action of antibiotics and other fungicides in lysing fungal mycelium may be a hastening of autolytic processes as a result of poisoning of the mycelium. Since these chemicals are not enzymatic in nature, their action could not be by direct dissolution of mycelium.

Zones produced by different isolates of *Streptomyces* spp. sometimes overlapped when streaked on agar in the same plate. Those areas that overlapped frequently contained less mycelium than did zones produced by the single isolates. This suggests that some isolates produced enzymes that initiated partial disintegration of the mycelium of the test fungus, whereas other isolates synthesized enzymes that lysed other constituents of the mycelium. This explanation would account for the additive effects observed.

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